DEVELOPMENT OF THE GEOGLOSSACEAE

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(WITH PLATES VIII–XII)

Introduction

Detailed knowledge of the ontogeny of Helvellinean fungi had its beginnings in the work of DITTRICH (7), whose paper on the complete development of *Mitrula phalloides* and *Leotia gelatinosa* (*L. lubrica*), with observations on other forms, has been the starting point for all subsequent life history studies in this group. This worker found that in the early stages of growth the ascocarps of *Mitrula* and *Leotia* possess a veil which, although evanescent, covers over the hymenium during the early part of their history, and thus renders their development endogenous. Heretofore the Helvellineae had been regarded as an essentially exogenous order, and SCHROETER (20) had separated them on this basis from the Pezizineae, in which group the hymenium was considered to be developed characteristically in a closed cavity which later opened. DURAND (11) confirmed and extended DITTRICH'S observations in the introduction to his excellent monograph of the Geoglossaceae. He states that he had observed the veil of *Mitrula phalloides* before learning of DITTRICH'S work. He had, in addition, noted the occurrence of a particularly well organized veil in *Microglossum viride*, and the conspicuous envelopes of *Spathularia velutipes* and *Cudonia lutea*. Of the latter he published beautiful photographs, illustrating these structures macroscopically.

The next contribution to this subject was a paper by MCCUBBIN (17) on the development of *Helvella elastica*. His work constitutes the first study in the family Helvellaceae, all the previous work having been confined to the Geoglossaceae. Here also it is stated that a membrane incloses the ascocarp in the younger stages of the form with which he worked. It is disorganized relatively early, and the last traces of it are cast off with the appearance of the paraphyses. BROWN (4) follows with an account of the development

of the ascocarp of *Leotia*, but, strange to say, he makes no reference to a veil, although his material apparently included very early stages of this form. His failure to mention the veil might be due to the fact that he was chiefly concerned with the origin of the asci and the nuclear phenomena connected therewith.

From these investigations it will be seen that in two of the three Helvellinean families, the Geoglossaceae and the Helvellaceae, an endogenous origin of the hymenium has been claimed. Until the publications of FITZPATRICK (**14, 15**), nothing was known of the conditions prevailing among the members of the remaining family, the Rhizinaceae. FITZPATRICK has now shown quite conclusively that *Rhizina undulata*, the type of the family, possesses no investment at any stage of its history, and that, therefore, not only is the hymenium "exposed from the first," but the ascocarp itself is naked, that is, "gymnocarpous."

In a preliminary communication the writer (**10**) briefly outlined his findings in an examination of four Geoglossaceous forms, namely, *Cudonia lutea*, *Spathularia velutipes*, *Trichoglossum hirsutum*, and *Leotia lubrica*. The veils of *Cudonia* and *Spathularia* were found to be present practically from the first. *Trichoglossum* proved to be devoid of all traces of a veil at every stage.. On the other hand, *Leotia lubrica* showed some slight traces of a veil, which might be comparable with the one claimed for it by DITTRICH, but the series of stages which the writer had under observation did not include ones sufficiently young to make diagnosis positive, and the question must still be regarded as an open one.

Turning to the question of the sexuality of the Helvellineae, there is still less to record. DITTRICH found no highly organized sexual apparatus in either *Mitrula* or *Leotia*, and he characterizes both forms as apogamous. The fertile hyphae here are differentiated from the vegetative threads at an early stage as conspicuous, deeply staining cells, with large single nuclei.

Similarly MCCUBBIN describes and figures the multinucleate, fertile elements of *Helvella* as having their origin in the vegetative hyphae, from which they spring at a later stage than is the case with *Mitrula* and *Leotia*. No sign of any body resembling an ascogonium was discovered at any stage.

On the other hand, BROWN (4) concludes that *Leotia* possesses an ascogonium which makes its appearance early in the history of the ascocarp. From this the fertile hyphae arise, and may be seen in succeeding stages, passing upward into the cap, where after much branching they reach the hymenium and form asci. This ascogonium was seen in but one plant, and was observed only in an empty and partly degenerated condition. The manifestations of sexuality in *Leotia*, therefore, cannot be said to have been established beyond the possibility of a doubt.

It is only in the work of FITZPATRICK (15) that we find a wholly satisfactory description of sexuality in any of the Helvellineae. This worker has given a very careful and detailed account of sexuality in *Rhizina*. In this form, while the ascocarp primordia are yet small, certain multicellular hyphae near the center are transformed into procarps. This transformation entails a great increase in diameter and in the number of nuclei in the cells, the assumption of an irregular coiling habit, and the development of a somewhat elongated terminal cell which may be considered a trichogyne. No antheridia are present, and the trichogyne is functionless. When maturity is reached, the centrally situated cells of the procarp proliferate ascogenous hyphae, and into these the nuclei from all parts of the procarp migrate, passing from cell to cell through pores which have previously been formed in the transverse septa. These nuclei migrate in pairs. Evidence of nuclear fusions in the ascogonial cells or in the ascogenous hyphae is entirely lacking, and neither conjugate nor simple divisions were observed to occur in the latter.

The conditions obtaining in the species discussed in this paper have already been summarized (10). It was pointed out that no evidence of the existence of any type of procarp bodies has been found in *Trichoglossum*, in which the ascogenous hyphae arise from threads of vegetative form adjacent to the hymenium. In *Cudonia* and *Spathularia* the structures interpreted as procarps arise at a comparatively late stage in the development of the ascocarp from threads differentiated while the fructification is yet very young. They are numerous, more or less irregular in form, and while in *Cudonia* very distinct trichogynes are organized, similar structures are absent from *Spathularia*. In *Cudonia* the cells of the procarp appear to be uninucleate at first, later becoming multinucleate,

while in *Spathularia* they are multinucleate from the beginning. The nuclei in the later stages of these bodies are arranged in pairs. Ascogenous hyphae take their origin in these procarps, and after engaging in crozier formation, give rise to asci.

Materials and methods

The material for this investigation was all obtained by collection, and was variously fixed, the fluids of Flemming and Carnoy, medium chrom-acetic acid and picro-sublimate, being used. It was imbedded in paraffin and cut in serial sections 2.5–10 μ thick. The usual staining methods were employed. Safranin and acid fuchsin proved to be the best general purpose stains, while Haidenhain's haematoxylin gave much the best results where details were required. In some cases the young fructifications sectioned *in situ* were stained by VAUGHAN'S (21) method for the differentiation of fungous hyphae and host tissues. This method gave very clear differentiation of the fungous tissue from the substratum, but had the serious disadvantage of impermanence.

As is not infrequently the case, the cells of certain tissues, while in a state of rapid growth and development, proved to be exceedingly difficult to differentiate cytologically, due to the density with which they are filled with deeply staining substances. As a result it has been impossible to answer some questions of nuclear behavior as fully as would be desirable. Otherwise the tissues appeared to be quite easily amenable to the usual microtechnical methods.

As already indicated, this paper deals in more or less detail with the four forms *Cudonia lutea, Spathularia velutipes, Trichoglossum hirsutum*, and *Leotia lubrica*. With the object of emphasizing the comparative aspects of the investigation, the contents are arranged in two parts, the first of which deals with the development of the ascocarp, with particular reference to the veil, and the second with the manifestations of sexuality in these plants.

I. Development of ascocarp

CUDONIA LUTEA

The earliest stage of *Cudonia lutea* of which we have knowledge is shown in fig. 3. At this stage the ascocarp is very minute, and measured but 84 μ from base to apex. It can be seen to consist of

loosely interwoven hyphae, which are obviously mycelial elements elevated to their present position chiefly by the upward growth of a central knot of conspicuous, deeply staining threads. The latter are of peculiar interest on account of the part they play in the development of the fertile system. At first sight, because of their marked differentiation from the other threads composing the young fructification, they might easily be taken for ascogenous hyphae, or even for ascogonia. At a later stage (fig. 6) the proliferations of these threads give rise to bodies of characteristic form and history, bodies that admit of one interpretation only, namely, that they are procarps. The first appearing threads must therefore have some significance other than this.

Clearly differentiated bodies are known to originate the procarps or corresponding bodies in various Ascomycetes. In *Ascobolus carbonarius* (DODGE 8) the procarps arise through the germination of asexual conidia borne on special mycelial branches. BROWN (3) found that in *Xylaria tentaculata* the Woronin hyphae are formed by the continued modification of threads that are differentiated at the center of the perithecium anlage while the latter is very young and deeply imbedded in the stroma. According to Miss DAWSON (6), a well differentiated thread appears in *Poronia punctata*, which at a later stage grows into a procarp.

The nearest approach to the first differentiated hyphae of *Cudonia* is to be found, however, in the disco-lichens. NIENBURG (18) gives a description of the development of the fruit bodies of four of these lichens, *Usnea barbata*, *Baeomyces roseus*, *Sphyridium byssoides*, and *Icmadophila aeruginosa*. It appears from his account that more or less well developed carpogonia arise in these forms, not from the ordinary hyphae of the ascomata in which they are found, but from threads which are differentiated at a considerably earlier stage. To these threads NIENBURG applied the name "Primordialhyphen" or "generativen Hyphen." In *Icmadophila* they appear before the fruit body has begun to rise above the surface of the thallus.

NIENBURG apparently was not very logical in his use of the terms "Primordialhyphen" and "generativen Hyphen." He employed the first in his study of *Usnea*, and the latter in studies of *Bae-*

omyces, *Sphyridium*, and *Icmadophila*. He drew no distinction between the so-called "Primordialhyphen" of *Usnea* and the "generativen Hyphen" of *Sphyridium* and *Icmadophila*. In *Baeomyces*, however, the carpogonia are so poorly defined that they are not separable from the "Primordialhyphen," so to the entire system in *Baeomyces* he extended the term "generativen Hyphen." Then, because of the evidently close natural relationship of *Sphyridium*, *Icmadophila*, and *Baeomyces*, he adopted the designation "generativen Hyphen" in connection with these forms. It is to be understood, therefore, that the "generativen Hyphen" of *Baeomyces* are not exactly homologous with those of *Sphyridium* and *Icmadophila*.

In the preliminary paper (10) the writer adopted the term "generative hyphae" rather than "primordial hyphae" to designate the pre-fertile threads of *Cudonia* and *Spathularia*. This was done because the Geoglossaceous forms appear to be more closely related to *Baeomyces*, *Sphyridium*, and *Icmadophila* than to *Usnea*. The occurrence of such a system of threads acting as precursors to the procarps is unusual, so far as the writer is aware, being found only in the disco-lichens, and so may be considered of sufficient importance to warrant emphasis.

The second developmental stage (fig. 4) shows a very definitely organized outer tissue, which has apparently been thrown up by the mycelial threads. By following through the series (figs. 4–7) this tissue may be seen to be identical with that later regarded as the veil. The veil in *Cudonia*, therefore, originates at a time when the ascocarp is nothing more than a cushion of mycelial threads with a core of generative hyphae, and can therefore be said to be present from the first. It is to be noted that at this stage a well marked clear zone separates the generative threads from the veil tissue. This clear zone contains only the remnants of the mycelial hyphae that once surrounded the generative threads. Fig. 5 illustrates the next developmental stage of *Cudonia*. Here the generative hyphae form an elongated mass of filaments of which the oldest are at the lower, and the newly formed elements, recently proliferated from them, are at the upper extremity. This upward growth of the generative threads has completely obliterated at the top the

clear space that once surrounded them on three sides, and at this point they are now in a subapical position, almost in contact with the veil tissue. Remnants of the clear space still remain on both sides, but it is rapidly being filled by the growth of fundamental tissue which has arisen from the mycelium, and which has pushed up before it the mass of generative hyphae. The intrusion of fundamental tissue from below has its beginning at an earlier stage, and indications of it may be seen on both sides at the base of the ascocarp in fig. 4. Judging from these two stages, it would appear that the most rapid formation of new tissue, at least during the early stages, takes place at the base rather than at the apex of the ascocarp.

The subapical position of the generative hyphae is retained as the ascocarp increases in size, until the apex broadens out to form the cap. These hyphae then pass into the cap, and there some of their branches are transformed into procarps. This transformation entails an increase in diameter, the assumption of an irregular coiling habit, and the development of a process which passes from the procarp more or less directly through the veil to the exterior. These processes are to be regarded as trichogynes. The history of the procarps will be discussed in greater detail subsequently. While at this stage, the upper portions of the cap are densely filled with generative hyphae, procarp complexes, and their proliferations the ascogenous hyphae, interwoven in an almost inextricable tangle (fig. 6).

It is important to note that until this time no sign of a hymenium has made its appearance. Very shortly after paraphysis formation commences, however, and following this the ascogenous hyphae proceed with the proliferation of asci. Paraphyses in *Cudonia* arise from undifferentiated vegetative elements. They are multiseptate, in this respect differing from the description given for this species by DURAND, and their cells are multinucleate. At first they are straight, but later become strongly recurved at the tips, which are also somewhat enlarged.

The veil still completely invests the entire ascocarp, including the hymenium. Over the hymenium it has become separated from the tissue of the subhymenial region, with, indeed, no attachment at all except at the edges (fig. 7). Nevertheless it does not cease

growth, but persists without rupturing, and keeps pace with the enlargement of the cap for considerable time. This power of independent growth is undoubtedly responsible for the persistence of the veil in this form, and is expressive of the distinct nature of the veil as an organ of the ascocarp and of its high degree of organization. Dehiscence finally takes place, by which time many of the asci are matured and their spores ready to be discharged. In the meantime, with the advent of the paraphyses the trichogynes disappear. The remaining portions of the procarps are visible for some time after, with cells empty or containing only a few strands and knots of plasm, evidently in a disorganized condition (figs. 12, 41). Finally practically all traces of them disappear.

Spathularia velutipes

Fig. 14 is a section of the earliest stage of *Spathularia velutipes* that has been studied. It can be seen to have been growing upon much rotted deciduous wood, and to have protruded above the substratum for somewhat less than 0.5 mm. From its size and organization it is evident that it represents a corresponding stage considerably older than the earliest *Cudonia* plants here figured. The interior of the young fruit body is composed of undifferentiated fundamental tissue, which is surrounded by a compact and well defined layer, which, as in *Cudonia*, is the investing membrane or veil.

By the use of suitable counter stains the veil of *Spathularia velutipes* may be sharply differentiated into two layers, an inner and an outer, the former compact and comparatively thin walled, and the latter looser and thicker walled. As the ascocarp increases in size, the outermost layer is split by tension into small adherent cell masses, which to the naked eye give the fruit body a velvety appearance. The power of independent growth is just as much a characteristic of the very persistent veil of *Spathularia velutipes* as it is of the membrane of *Cudonia*. In contrast to the condition of *Cudonia* at this stage, however, the interior of the ascocarp is still undifferentiated, and no threads corresponding to the generative hyphae of that form are yet visible.

By the time the ascocarp has attained the stage represented in fig. 15, conspicuous hyphae have made their appearance, and are situated as the generative hyphae come to be in *Cudonia*, just

behind the apex. These conspicuous threads in the young *Spathularia* plant are also generative hyphae, for, as will shortly be seen, certain of their proliferations at a very much later stage are transformed into procarps. As the young ascocarp increases in size the pointed apex becomes broadened out, and the generative hyphae are distributed in an arch below it. This arch widens in concert with the gradual expansion of the apex into a somewhat globular cap, which as time goes on assumes the spatular shape characteristic of the mature plants of the genus (figs. 16, 17).

The expansion of the apex into the cap is marked by the appearance of paraphyses. The paraphyses of *Spathularia* arise from vegetative hyphal threads, the end cells of which become somewhat club-shaped. Various students of other species, as McCUBBIN (17) and BROWN (4), have found paraphyses arising from specialized storage cells. This does not occur here, the cells from which the paraphyses arise being insufficiently differentiated to be regarded as storage bodies. In form, septation, and nucleation the paraphyses of *Spathularia* resemble those of *Cudonia*.

While the paraphyses are still young, procarps become evident for the first time. They are scattered about in irregular fashion through those portions of the cap containing the generative hyphae, and are complexes conspicuous for their size and staining qualities (figs. 19–21, 43–45). An examination of all stages after this until the veil is shed and spores are being cast reveals their presence continuously. They vary in shape; some appear as coiled structures, some are immensely swollen, and others take the form of chains of cells. These bodies arise from the generative hyphae just as was the case in *Cudonia*, and from them ascogenous hyphae proceed directly to the hymenium, where in turn they may be seen giving rise to asci. Further growth results, as in the case of *Cudonia*, in the introduction of no new tissues or organs, but in the mere expansion of the ascocarp. The hymenium reaches a fairly well advanced state of maturity before the veil bursts. The observations of the writer have not established any regularity in the method of rupture, but DURAND states that "the veil seems to rupture by a crack running around the plant just above the stem," and exhibits photographs which illustrate his point.

Trichoglossum hirsutum

The very young fructification of *Trichoglossum hirsutum* shown in fig. 22 measures 175 μ in height, and is of simpler structure than the corresponding stages of either *Cudonia* or *Spathularia*. It is composed of compactly intertwining threads, the walls of which are comparatively thick and very dark. The hyphae all appear to be alike except for some, which, situated near the periphery and at right angles to it, extend beyond the ascocarp as straight, sharp pointed, unicellular hairs of greater diameter and thicker walls than the other threads. These setae correspond to similar ones described by Brown in *Lachnea scutellata* (5), and by Fitzpatrick in *Rhizina undulata* (14). The setae of *Trichoglossum* are differentiated somewhat earlier than in *Rhizina*, but do not contain a glutinous substance such as is found in those of the latter form. The setae in *Trichoglossum* appear to be continuously produced over all peripheral parts of the ascocarp as it grows, but those that are earlier formed persist for some time, and come to be imbedded deeply in the new tissues that grow up around them. This is particularly evident in the hymenial region, where such growth is rapid (figs. 25, 26).

Paraphysis formation commences reatively early at the apex of the ascocarp, before it has begun to broaden out to form the cap (figs. 24, 25). The paraphyses are large, unbranched, multiseptate threads, the cells of which are usually uninucleate, but apparently may come to contain several nuclei in exceptional cases. They are strongly coiled at the tips.

Shortly after the paraphyses are organized, broadening of the apex of the ascocarp begins, and the hymenium extends over the entire surface of the globular cap as it takes shape and expands into the mature condition.

From the figures it may be seen that the hymenium is of undoubtedly exogenous origin, and, moreover, the ascocarp itself is clearly gymnocarpous. Throughout the entire series of stages careful examination has failed to reveal the presence of an investment of any kind. *Trichoglossum hirsutum*, therefore, adds one more species to those Helvellineae the hymenium of which is exposed from the first and the fructification gymnocarpous.

The vegetative threads in a well developed ascocarp of *Trichoglossum hirsutum* constitute a loosely interwoven tissue in which the individual cells of the threads are clearly distinguishable, even in thick sections. The threads pass with more or less uniformity from the base to the apex of the ascocarp. Since they are thick walled and consist of uninucleate cells, it is not difficult to see that hyphae of any other type are absent. The paraphyses make their appearance at an early stage (fig. 24), but ascus formation does not commence until the cap is well shaped. Then, just beneath the bases of the paraphyses in the subhymenium, there appear thin walled hyphae which, unlike the vegetative threads, form a very densely interlaced tissue. These constitute the ascogenous system of *Trichoglossum*. It is certain that no bodies such as the generative hyphae and procarps of *Cudonia* or *Spathularia* stand out as specific organs, nor is it possible to distinguish the homologues of procarps as distinct from the ascogenous hyphae.

The ascogenous hyphae are short, since they are differentiated in such close proximity to the hymenium. Asci arise from them in the usual manner. The formation of an ascus is preceded by the inversion of the tip of an ascogenous hypha to form a crozier, the ascus growing out of the penultimate cell. The asci are very large, with dense protoplasm, and, especially after the dark multiseptate spores are mature, form very conspicuous objects in the hymenium (fig. 26).

LEOTIA LUBRICA

Since material of this form had been collected, and since previous investigators are not in complete agreement on two important points in the life history, it was thought advisable to examine the material with a view to the possibility of shedding some further light on these points. The questions to which reference is made are those of the presence of the veil and the origin of the ascogenous hyphae, and attention was confined to these questions alone.

Two specimens at approximately the same well advanced stage showed irregular tissue fragments overlying parts of the ascocarp and the hymenium (figs. 27, 28). These specimens were not the youngest of the fruit bodies examined. In no others, however, whether younger or older than these, were any similar evidences

seen. These fragments are composed of interwoven threads more or less degenerated in appearance, and in one case still connected with one another by a few hyphae, which give them the appearance of having been torn apart by the growth of the ascocarp (fig. 27). DITTRICH (7) describes the veil of *Leotia* as having a ground substance of gelatinous material formed by the progressive inward swelling ("Verquellung") of the peripheral hyphae. In this matrix are imbedded threads which are rather compactly interwoven. Apparently, therefore, so far as structure is concerned, the tissue fragments observed correspond fairly well to the veil tissue of *Leotia* as described by DITTRICH. Had very young ascocarps been available, and had such tissue been found covering them, particularly as entire structures, DITTRICH'S view that *Leotia* is at first angiocarpous would have been substantiated. On the other hand, had the material included a satisfactory series of very young stages which did not show traces of a veil, the writer would have felt justified in denying its presence in *Leotia lubrica*. The youngest stages available, however, were advanced sufficiently to show a well differentiated cap and young paraphyses, and consequently were considerably older than the youngest stages which BROWN, and probably also DITTRICH, had under observation. Under these circumstances the writer does not feel that the evidence is sufficient to lend conclusive support to either view. It would seem best, therefore, to regard the question as still open.

SIGNIFICANCE OF VEIL IN HELVELLINEAE

Ever since the appearance of DITTRICH'S (7) original paper on this subject, each succeeding investigation has served to emphasize the inadequacy of the distinction drawn by SCHROETER (20) between the Helvellineae and the Pezizineae. According to this classification, in the former group the hymenium is formed upon the surface of the ascocarp, and therefore is freely exposed from the first, while in the latter the hymenium is originally inclosed. SCHROETER'S fundamental idea appears to have been that in the Pezizas the hymenium is formed within the ascocarp in an inclosed depression, while in the Helvellas it appears upon a flat or convex surface, not closed in.

The discovery of the veil introduces a new morphological feature not contemplated in SCHROETER'S scheme. The value of the veil as an important taxonomic criterion depends, not so much upon the fact that it may at first inclose the hymenium, as upon its nature as an organ of the ascocarp. If it were simply the roof of a depression, comparable with the roof that covers over the young hymenium of a *Peziza*, and which breaks away to form the edge of the cup, then the Helvellinean forms in which it occurs would plainly be only modified Pezizas. But if, as seems to be the case, it were a distinct envelope which covers over the entire ascocarp, and perhaps incidentally also the young hymenium, then so far as we know it is a feature not represented in the Pezizineae, with the possible exception of the as yet uninvestigated Helotiaceae and Mollisiaceae.

In reviewing the conditions prevailing in the Helvellineae, it is found that the veil is absent in the only member of the Rhizinaceae which has been studied, it is present in the only member of the Helvellaceae of which we have knowledge, while at the present time the Geoglossaceae are about equally divided. In the last named family the following species may be considered as angiocarpous: *Mitrula phalloides*, *Leotia lubrica* (DITTRICH 7); *Microglossum viride* (DURAND 11); *Cudonia lutea*, *Spathularia velutipes* (DURAND 11, DUFF 10). The gymnocarpous members appear to be *Geoglossum glabrum*, *G. difforme*, *Trichoglossum velutipes* (DURAND 11); and *Trichoglossum hirsutum* (MCCUBBIN 17, DUFF 10). It may be noted that the gymnocarpous species are all closely related, belonging to genera that were at one time comprised in the single genus *Geoglossum*, while the others, with the exception of *Microglossum viride*, belong to less closely related genera. This means, so far as our evidence goes, that the *Rhizina* and *Geoglossum* groups are typically gymnocarpous, while the remaining Helvellineae are angiocarpous.

In searching for a structure in other Ascomycetes which might be homologized with the Helvellinean veil, we find one in the *Baeomyces* group of the disco-lichens. Some such relationship had already suggested itself to DITTRICH, who, after emphasizing other points of similarity, goes on to say that in *Baeomyces* the upper portions of the thallus which overlie the anlage of the fruit body, and which later surround the young fructification, seem to assume the

"functions" of a veil. Although he does not say so explicitly, it is a fair inference that DITTRICH considered this thalloid investment of the young *Baeomyces* fructification and the inclosing membranes of *Mitrula* and of *Leotia* homologous, for he implicitly adduces this as evidence of the Geoglossacean affinities of *Baeomyces*. DITTRICH's conclusions were based upon the description of the development of the fructification of this lichen given by KRABBE (16). NIENBURG (18) re-examined more recently some of the forms with which KRABBE worked, and the results recorded in his paper present even more interesting parallelisms to the conditions prevailing among some of the Geoglossaceae.

In *Icmadophila aeruginosa* the covering of the ascocarp is more distinct and more persistent than in *Baeomyces*. Figs. 1 and 2, taken from NIENBURG, represent sections of very young fruits of the former species. Here it may plainly be seen that the superficial layer of the thallus grows into an envelope for the young ascocarp and incloses it until it has attained considerable size, after which the growth of the ascocarp becomes too rapid for the extension of the epithecial tissue, which then ruptures. The fructification does not subsequently become covered with any other tissue which might be compared to a veil, and the hymenium therefore develops exogenously. As has already been indicated, however, the point of importance in this connection is not the origin of the hymenium, but only the occurrence of an envelope which incloses the entire ascocarp, and which constitutes a morphologically distinct organ of the fruit body. In the opinion of the writer, therefore, the envelopes of *Baeomyces* and *Icmadophila* are strictly comparable and homologous with those of the Geoglossaceae. From this it follows that in any natural scheme of classification some lichens, at least those of the *Baeomyces* group, must be brought in with the angiocarpous Geoglossaceous forms, as also possibly some members of the Helotiaceae.

II. Sexuality

CUDONIA LUTEA

It is in the youngest stages of *Cudonia lutea* that we find the beginnings of the hyphal system destined finally to give rise to asci. As we have seen, here there are differentiated from the

mycelium certain threads which have been called generative hyphae, and which are the most prominent central components of the simply organized fructification. These threads are clearly differentiated in the stained preparation from the rest of the ascocarp. So vigorously do they react to stains that all details of cell structure are obscured, even when nuclear stains are applied.

The generative hyphae grow upward as the ascocarp increases in length, remaining subapically situated. When the upper part of the fruit body is differentiated into the cap these hyphae make their way into it, and in that position certain branches of this system appear that are enlarged, irregularly coiled, and deeply staining (fig. 6). These are the procarps. The procarp coils are continued upward by processes which can only be regarded as trichogynes (figs. 6, 8, 9). These are multiseptate and follow a more or less direct course toward the exterior. Where the procarps lie in a suitable position the trichogynes may be followed for their whole length, and may be seen to penetrate the veil tissue and to reach the exterior. Sometimes the procarps are deeply imbedded, however, and in these cases it is impossible to follow the trichogynes as far as the surface of the ascocarp.

The number of procarps formed in this manner in a single fruit body appears to be very large, although it is not possible to count them, on account of the way in which they are interlaced and because of the presence of masses of generative and ascogenous hyphae. Some idea of their number may be conveyed, however, when it is known that as many as seven distinct trichogynes have been counted in a single longitudinal section 10 μ thick through a region of the cap in which they are numerous. Their distribution is irregular. Some of them are formed closely under, or even partly imbedded in the veil tissue, while others are situated at a distance from the surface in the direction of the center of the cap (figs. 6, 8, 9). Sometimes they are localized in one or two restricted portions of the cap, being entirely absent from others.

Fig. 11 is a portion of an unusually loosely organized procarp. This section does not contain any of the upper portion of the procarp, but it shows most of the lower part and the generative hypha from which it arose. The latter follows a somewhat

straighter course than is usual with these threads, and hence its relation to the coil is rendered very obvious. The generative hypha is more deeply stained than the surrounding vegetative threads, while the procarp coil itself is quite opaque. Figs. 32–35 represent other procarp bodies at approximately the same stage of development as that of fig. 11. Most of these figures are taken from sections cut longitudinally through the ascocarp, but fig. 34 represents portions of a procarp found in two contiguous sections of a series taken in a transverse plane. These illustrations give some idea of the diversity of form displayed by these structures. They vary from an almost straight, slightly twisted series of cells, to coils such as that of fig. 11. These illustrations also show the relationships of the procarps and trichogynes. This relationship is evident in fig. 6. Two procarps and their trichogynes appearing in this section are shown enlarged in fig. 8, where, however, on account of the thickness of the section and the meandering habit of the threads of the procarp and trichogynes, not all of these structures can be obtained in sharp focus at the same time. The protrusion of the trichogyne from the surface of the ascocarp is shown well in fig. 13, which is the first section of a series through the cap of an ascocarp at this stage. The exposed tip of a trichogyne, cut off with the first section, is clearly shown, several of its cells being visible.

No structures have been found that could be regarded as spermogonia, and no bodies that correspond to spermatia. Furthermore, the writer has no evidence that the trichogynes functioned in any way, and since they disappear very soon, is inclined to regard them as vestigial organs in *Cudonia*.

As may be seen from the illustrations, the procarps are very deeply staining bodies, and in their earlier stages it is impossible to differentiate them cytologically. Nevertheless here and there an occasional cell may be seen to contain what is doubtless a single nucleus, while a smaller number contain two (figs. 32–35). Thus what slight evidence there is points to the original condition of these bodies as being uninucleate. A little later on, however, they become multinucleate. This takes place soon after the appearance of the paraphyses, and is preceded by the disappearance of the trichogynes. At this stage the remaining cells of the procarps become

more distended, and their contents do not stain quite so densely, and thus cell structures can be distinguished with a greater degree of certainty. How the originally uninucleate cells become multinucleate is not known. The nuclei are small, and are frequently unequal in size. They often appear to lie in pairs (figs. 36, 37, 39, 40).

While at the multinucleate stage, or even before they can be seen definitely to be multinucleate, the procarp cells form branches which bud out from them (figs. 36, 38, 40). These branches are ascogenous hyphae, and can be recognized directly as such from the fact that in well advanced specimens in which the formation of asci is just beginning they have been seen passing from procarp complexes, which are usually empty and degenerated at this stage, to the hymenium, and there giving rise to asci (fig. 10). Ascogenous hyphae may be seen passing from an old procarp complex to the hymenium, which is just being differentiated (fig. 10). In the subhymenium they are in direct connection with cells undergoing crozier formation preparatory to the formation of asci. The cells of the ascogenous hyphae are usually multinucleate, but may be binucleate.

The proliferation of one or more asci from an ascogenous hypha by the formation of a crozier, with its four nuclei and various evolutions, has been described by numerous workers, and need not be detailed again. It will be sufficient to say that in *Cudonia* the penultimate cell usually grows out into an ascus, but a succession of croziers may be formed, and there may be a fusion of the ultimate and the antepenultimate cells, followed by the formation of an ascus or of another crozier. Several croziers may be formed from a single cell of an ascogenous hypha, and when all these are confined to the distal end of the cell a peculiar candelabra-like arrangement of asci results.

The two nuclei which find their way into the young ascus fuse at once. The fusion nucleus enlarges as the ascus grows, and finally assumes very large proportions. Divisions of the nucleus begin when the ascus has reached about half its ultimate size, and these divisions succeed one another very rapidly. When eight daughter nuclei have been formed the spores are delimited.

Spathularia velutipes

The origin of the generative hyphae in *Spathularia* has already been described. Mention has also been made of the fact that they come to occupy such a position in the ascocarp that when the hymenium has been formed they will lie just beneath it. Apart from their somewhat larger size, conspicuous staining qualities, and restricted position in the ascocarp, the generative threads do not appear to differ from the vegetative hyphae. The cells of both contain from one to several nuclei.

After the paraphyses have been formed, procarps grow out from some of the cells of the generative hyphae. The procarps are of relatively large proportions and become very strongly chromophilous. They are curious conspicuous complexes of hyphae. Attention has already been called to them, and some of them are illustrated in figs. 19–21 and 43–45. They appear to exhibit no uniformity of structure, but their cells are all multinucleate, and frequently distinct pairing of nuclei is observable (figs. 43–45.)

Ascogenous hyphae arise from these procarps. This is very easily demonstrated where the hyphal complex lies particularly close beneath the hymenium, and where the ascogenous hyphae pass directly into the hymenium. Under these circumstances the ascogenous hyphae take on something of the staining qualities of the cells from which they arise, and they may be traced with ease into the hymenium, and there may be seen in connection with asci. Fig. 21 is a plexus of such ascogenous hyphae arising from a group of large cells, some of which are visible. This large group may be traced through a series of sections 7.5 μ in thickness. The origin and termination of these threads are not visible in all sections, but by following through the series their connections in both directions are easily ascertained.

Fig. 19 affords another illustration of this type, showing two enlarged procarp cells, somewhat depleted of their contents, one containing a single nucleus and the other two nuclei. From these cells deeply staining ascogenous hyphae are to be seen passing outward, following a very irregular course to the hymenium, and there in direct union with young asci. The group of uninucleate asci of about the same age shown in this figure appears to have arisen

from ascogenous hyphae originating in the same procarp cells. More frequently, however, the hyphae passing from the procarps do not retain their staining qualities sufficiently long to make it possible to follow them to the hymenium, especially when they are much branched or when they follow a devious route. The cells of the ascogenous hyphae are multinucleate, and the nuclei appear to be paired (fig. 45).

McCUBBIN, who described bodies in the ascoma of *Helvella elastica* which bear considerable resemblance to those at present under discussion, took the view that they are merely storage organs. In addition to ascogenous hyphae McCUBBIN claims to have found paraphyses arising from these so-called storage cells. Judging from his figures, however, the paraphyses arising in this manner are so far from typical that it may be questioned whether they are actually paraphyses. Since the "storage bodies" of *Helvella* resemble the procarps of *Spathularia* in being multinucleate, in having the nuclei arranged in pairs, and in producing ascogenous hyphae, it would seem to the writer that they might also be interpreted as sex organs. FITZPATRICK (15) has already expressed this opinion. He states that "several significant facts would seem to indicate that at least part of these 'storage bodies' constitute some type of sexual apparatus, particularly the statement that they are sometimes found giving rise to ascogenous hyphae." The ascogenous hyphae of *Spathularia* behave as in the case of *Cudonia* in the evolution of asci. The description given for the latter form may be taken to include them both.

LEOTIA LUBRICA

According to DITTRICH, the fertile threads of *Leotia lubrica* are differentiated from vegetative hyphae at an early stage in the history of the ascocarp. While they do not appear to be easily traced in intermediate stages, they become conspicuous again as maturity approaches, this time in the cap, where, lying just beneath the hymenium, they give rise to asci. Although DITTRICH does not figure the youngest stage in which they are visible, as he does in the case of *Mitrula phalloides*, he says that these two forms are much the same in this regard. If so, the fertile hyphae appear at a very early stage, indeed, one almost comparable with the youngest *Cudonia* plants described in this paper.

On the other hand, BROWN (4) has figured the remains of what he interpreted to be an ascogonium, a structure found by him in one case only, at the base of an older ascocarp than the youngest with which DITTRICH presumably worked. The ascogenous hyphae pass up from the ascogonium, and may be traced to the cap, where they become much branched, finally finding their way to the hymenium. The writer's material did not include stages young enough to make possible a direct confirmation of either of these statements, but it did show hyphal complexes just previous to the formation of the first asci strikingly suggestive of those already described for *Spathularia*.

At this period the ascogenous hyphae appear under the hymenium as deeply staining threads in various stages of crozier formation and ascus proliferation, and since they are not numerous as yet, they are easily distinguished and recognized. Beneath them in the cap may be seen at the same time very conspicuous groups of greatly enlarged cells. Some of these enlarged cells are quite empty, some contain light vacuolated protoplasm, and others are densely filled with deeply staining contents, identical in appearance with those of the ascogenous hyphae. These enlarged cells are usually rounded, occurring singly or in groups, but they may assume any of a great variety of forms. Their distribution in the ascocarp follows no regular arrangement whatever. They contain from one to several nuclei which are variable in size and sometimes may be very large (figs. 47–53). From these large cells ascogenous hyphae arise. This is readily demonstrated wherever they occur close to the hymenial region.

BROWN has described what he regards as storage cells in the ascocarp of *Leotia*. Of these he says: "While the hymenium is being differentiated some of the vegetative hyphae give rise to large storage cells. . . . . These large storage cells are formed in rows and give rise to paraphyses. The storage cells are at first multinucleate, but the nuclei usually fuse as growth proceeds." He affirms that the nuclei of these cells, where they are more than one, may be very unequal in size, and the fusion nuclei are sometimes of extraordinarily irregular form. The writer has not been able to find storage cells which exactly correspond to those described by BROWN. The only large unusual cells noted were those

already described, and it is believed that these are BROWN'S "storage cells." Careful attention was given to the possibility of paraphyses arising from these cells, but in the many sections examined for the purpose of confirming this observation, not a single clear case of the origin of paraphyses in these bodies was found.

The fact that ascogenous hyphae arise from these special cells in *Leotia* makes it necessary to consider the possibility that they represent some form of sex apparatus. Obviously, before this interpretation can actually be put upon them, it will be necessary to re-examine stages comparable with the youngest which BROWN studied in order to make sure whether or not an ascogonium occurs at the base of the young ascocarp. If there be no ascogonium there, *Leotia* would then resemble *Spathularia* in its manifestation of sexuality.

SEXUALITY IN HELVELLINEAE

The phenomena of sexuality in the Helvellineae bring forward some interesting questions. In the first place, the large number of procarps occurring in these Geoglossaceous forms is noteworthy; the overwhelming majority of Discomycetes possess but a single one. FITZPATRICK (15) drew attention to this point in his discussion of *Rhizina undulata*, which form is characterized by the production of several of these bodies. This investigator also indicated that while this character tends to separate the Helvellineae from other Discomycetes proper, it brings them closer to the disco-lichens, which are notable for their compound apothecia. FITZPATRICK quotes a number of lichen forms of which this is true, and to these may be added those which constitute the subject of NIENBURG'S (18) researches, namely, *Usnea barbata*, *Baeomyces roseus*, *Sphyridium byssoides*, and *Icmadophila aeruginosa*. It may be mentioned also that in those species in which the procarp is not obviously greatly reduced, for example in *Rhizina undulata* and in *Cudonia lutea*, it is of the same general form as that which characterizes lichens, that is, it consists of a modified, more or less coiled multicellular hypha the terminal portion of which constitutes a trichogyne. The trichogyne is unicellular in *Rhizina* and multicellular in *Cudonia*.

Another feature in common with the lichens is the fact that the procarps of the Helvellineae are not "initial organs" arising from the mycelium and later becoming surrounded by the tissue of the ascocarp, as they are in *Ascobolus*, etc., but are formed within an already well developed ascocarp. This is true of *Rhizina*, in which the fructification begins as a "wholly undifferentiated button of mycelium." It is not until the ascocarp has attained a diameter of about 1 mm. that the procarps appear. They then arise by the differentiation of certain centrally situated hyphae of the fruit body. In *Cudonia* the procarps arise at an even later stage, that is, after the ascocarp has become differentiated into cap and stem, while those of *Spathularia* are delayed still further in their appearance.

There is still another point of resemblance between these Geoglossaceous fungi and the disco-lichens in their common possession of a feature noted by NIENBURG (18), namely, that the procarps are offshoots of a unique hyphal system (designated by him "generativen Hyphen"), which makes its appearance at an early stage. NIENBURG shows that in *Icmadophila aeruginosa* the "generativen Hyphen" appear as deeply staining threads in the young ascocarp anlage before it becomes erumpent. As the fructification increases in size these hyphae proliferate to form scattered knots of threads, which are connected together, having a common origin, by "Verbindungshyphen." The latter are evidently a part of the system of "generativen Hyphen." These knots become transformed into procarps, and distinct trichogynes are formed which penetrate the tissues of the ascocarp and project into the air. Although spermatia frequently become attached to the trichogynes there is no conclusive evidence that they are functional, or that a process of fertilization takes place. Many of the procarps disorganize without producing ascogenous hyphae, but those that survive act as the source of these threads.

In *Sphyridium* the first elements of the pre-fertile system, the "generativen Hyphen," appear at a somewhat later stage than in *Icmadophila*. In this form they grow into a series of very clearly defined nests of hyphae, which, as in *Icmadophila*, are connnected together by "Verbindungshyphen." The growth of the vegetative

hyphae carries these knots to the upper portion of the ascocarp near the surface and separates them from one another. The ascogenous hyphae arise from these structures. They evidently represent some form of sex apparatus, but because of their lack of conventional trichogynes and their irregular habit, NIENBURG hesitates to give them the full status of typical "carpogones." He says: "Ich glaube deshalb, dasz regelrechte Trichogyne bei Sphyridium nicht mehr angelegt werden, sondern dasz wir hier reduzierte Gebilde vor uns haben, die wahrscheinlich früher als Empfängnisapparate gedient, heute aber diese Funktion aufgegeben haben."

In *Baeomyces* the young fructification is sunken in the thallus and is distinguished as a mass of hyphae more closely interwoven than those of the thallus. At the center of this mass appear certain enlarged, deeply staining threads which are the "generativen Hyphen," and which are the source of the ascogenous hyphae. NIENBURG'S uncertainty in the interpretation of these bodies is indicated in the following quotations: "So ist zu vermuten, dasz auch hier auf sehr frühem Stadium, Askogone als Verzweigungen der vegetativen Hyphen gebildet werden, die man nur mit unsern Mitteln von den übrigen Gewebe—dem askogenen wie dem vegetativen—nicht deutlich unterscheiden kann. . . . . Vielleicht sind die dunklen Hyphen in den Knäueln der Fig. 17 solche Askogone, vielleicht sind es aber auch schon askogene Hyphen." He decides that they may represent carpogonia, but if so, that they are very much reduced in *Baeomyces*, which he regards as an apogamous form. It is to be noted, however, that ascogenous hyphae still arise from these bodies even if they are structurally reduced.

These brief reviews indicate how close is the correspondence in the history of the fertile system between some of the disco-lichens and of the Geoglossaceae. In both groups the procarps are numerous; they have, in general, the same type of structure; and in both are formed at a more or less advanced stage from a system of threads which makes its first appearance while the ascocarp is very young.

It is interesting that in both groups parallel series are found showing comparable stages in structural reduction. In the lichens we have a graded series in *Icmadophila*, *Sphyridium*, and *Baeomyces*.

In *Icmadophila* the procarps are slightly if at all reduced, in *Sphyridium* they have lost the trichogyne and become somewhat irregular in form, while in *Baeomyces* it is questionable whether such structures can be said to occur at all. Among the Geoglossaceae we have *Cudonia* illustrating a condition entirely comparable with that of *Icmadophila*, while *Spathularia* resembles *Sphyridium* in the much-reduced nature of its procarps. In *Trichoglossum* the sex organs do not stand out as morphologically distinct structures. That these stages in reduction give a picture of the direction of evolution of sexuality in both groups is the opinion of the writer. Beginning with a form of procarp in which a true process of fertilization through the medium of the trichogyne took place, the evolution of these plants seems to have been marked by the successive suppression of the male organ, the trichogyne, and finally the procarp as a distinctive morphological body.

The facts observed in the Geoglossaceous forms examined point in a contrary direction to the opinion held by BLACKMAN and WELSFORD (1), as stated in their paper on *Polystigma rubrum*. They maintain that in all cases where a degenerate ascogonium occurs, the ascogenous hyphae will probably be found to originate independently of this organ. Their own recorded observation that the well organized procarp of *Polystigma rubrum* takes no part whatever in the formation of ascogenous hyphae lacks confirmation. Indeed, NIENBURG (19), in a more recent work on this species, maintains that ascogenous hyphae do originate in the procarp. BROOKS's (2) similar contention with regard to the part played by the procarp of *Gnomonia erythrostoma*, and FISCH's (13) statement on Woronin's hypha in *Xylaria polymorpha*, are offset by H. B. BROWN's (3) observation that the cells of the Woronin hypha of *X. tentaculata* actually do become ascogenic.

The parallelism shown to exist between Geoglossaceous and disco-lichen forms must surely be significant of phylogenetic relationship. The question of the relationship of these groups is of practical interest, in view of the conviction on the part of certain botanists that the lichen genera should be distributed among those of the fungi proper. It is recognized by many who favor such a procedure that the history of the reproductive tracts must ulti-

mately be the basis of any such reclassification. The following quotation from FINK (12) exemplifies this view: "The classification of the fungi is really based in the main upon the morphological relationships of the reproductive areas, and in the lichens also these relationships surely have a greater importance in classification than the form-relationships of the thallus." This being so, such correspondences as have been disclosed here should indicate a basis of relationship between the Geoglossaceae and the *Baeomyces* group of the disco-lichens. This evidence of relationship is made stronger by the homology shown to exist between the veils which inclose the ascocarp in many of these two groups. The further extension of this relationship should aid materially in the problem of classification, and should give direction to our ideas concerning the affinities of these two interesting groups of plants.

Summary

1. The development of the ascocarp in *Cudonia lutea* and in *Spathularia velutipes* is notable for the appearance of an inclosing membrane which covers over the entire fructification, and which constitutes a morphologically distinct organ of the fruit body. Very early stages of *Cudonia* show its presence, and it is one of the first organs of the ascocarp to be differentiated. *Trichoglossum hirsutum* lacks entirely any such investment, and the ascocarp is therefore gymnocarpous. *Leotia lubrica* shows some evidences of possessing an evanescent veil, but this is a point which requires further elucidation.

2. The value of the Helvellinean veil as a systematic criterion depends more upon the fact that it is a morphologically distinct organ inclosing the entire ascocarp than that it may result in an endogenous development of the hymenium. This being so, the closest homology to the Helvellinean veil is to be found, not in the Pezizineae, but in the *Baeomyces* group of the disco-lichens. This is adduced as evidence of relationship between these groups.

3. The fertile systems of *Cudonia lutea* and *Spathularia velutipes* appear at an early stage in the history of the fructification as threads which have been called "generative hyphae." These hyphae proliferate with the other tissues of the ascocarp, and at

a later stage give rise to the procarps. The procarps of *Cudonia* are irregularly coiled, and are provided with multiseptate trichogynes which pass outward and protrude through the veil. The nuclear history is not fully known, but the original condition of the cells of the procarp seems to be uninucleate, later becoming multinucleate, and at this stage giving evidence of nuclear pairing. Ascogenous hyphae arise from the cells of the procarps, after which the procarps become emptied of their contents and finally disappear. The procarps of *Spathularia* appear somewhat later than in *Cudonia*, and are formed close to the hymenium. They are irregular, do not possess trichogynes, and are obviously reduced in structure. They are multinucleate throughout their history, and the nuclei are paired. Ascogenous hyphae arise from them.

4. In *Trichoglossum hirsutum* there is no structural differentiation of sex organs. The ascogenous hyphae arise from threads which do not differ in form from vegetative ones.

5. In *Leotia lubrica* bodies resembling in some respects the "storage bodies" described by BROWN for the same form were found to produce ascogenous hyphae. On this account it is suggested that they may represent sex organs, that is, they may be degenerate procarps.

6. It is pointed out that there is a very close correspondence in the history of the fertile systems of the Geoglossaceae and of certain disco-lichens of the *Baeomyces* group. This is cited as further evidence of relationship between them.

7. It is suggested that the progress of evolution in these plants has been from a type in which fertilization took place through the agency of the trichogyne, and has been marked by gradual reduction of the sex organs.

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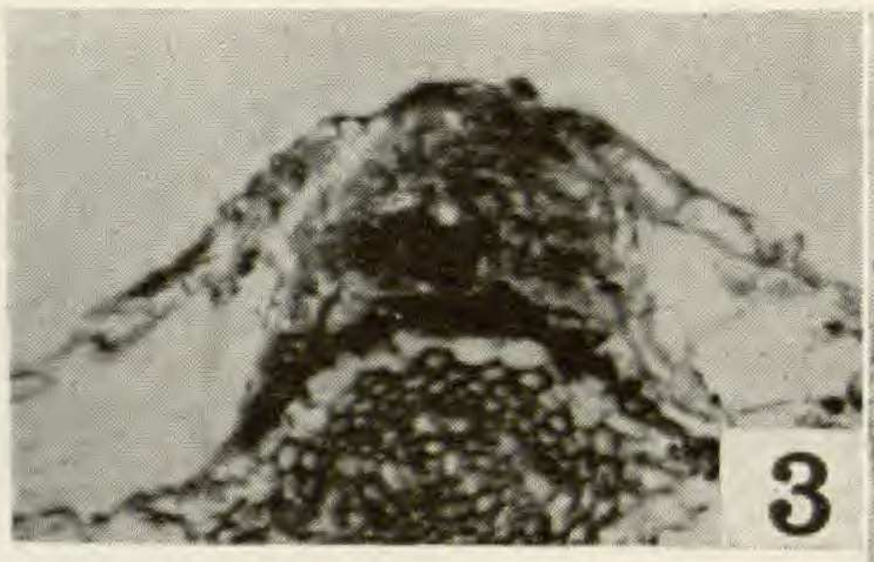

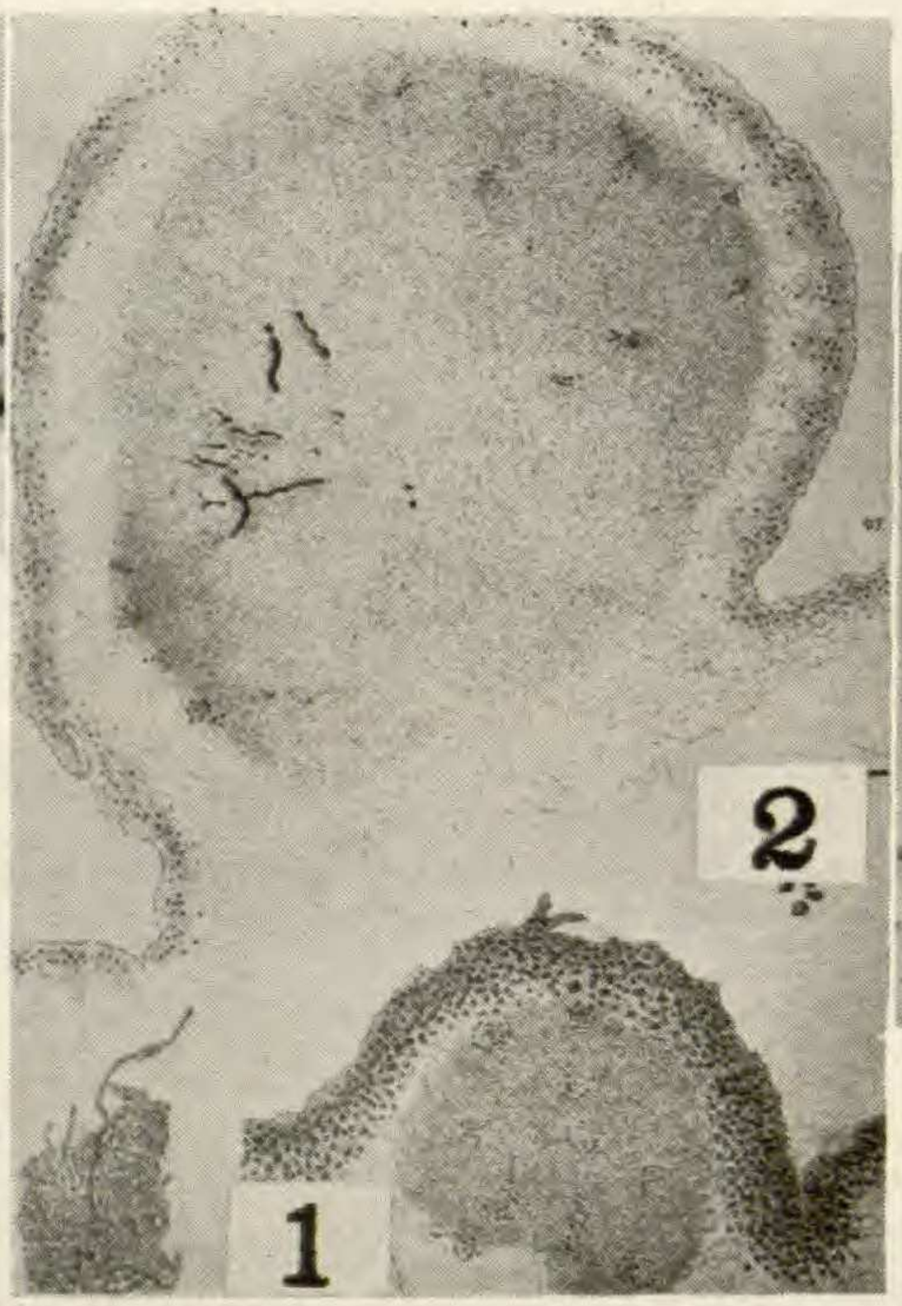

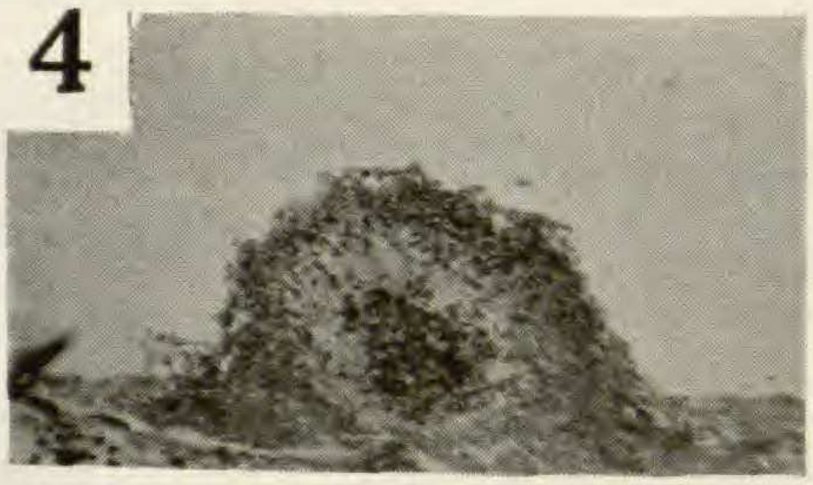

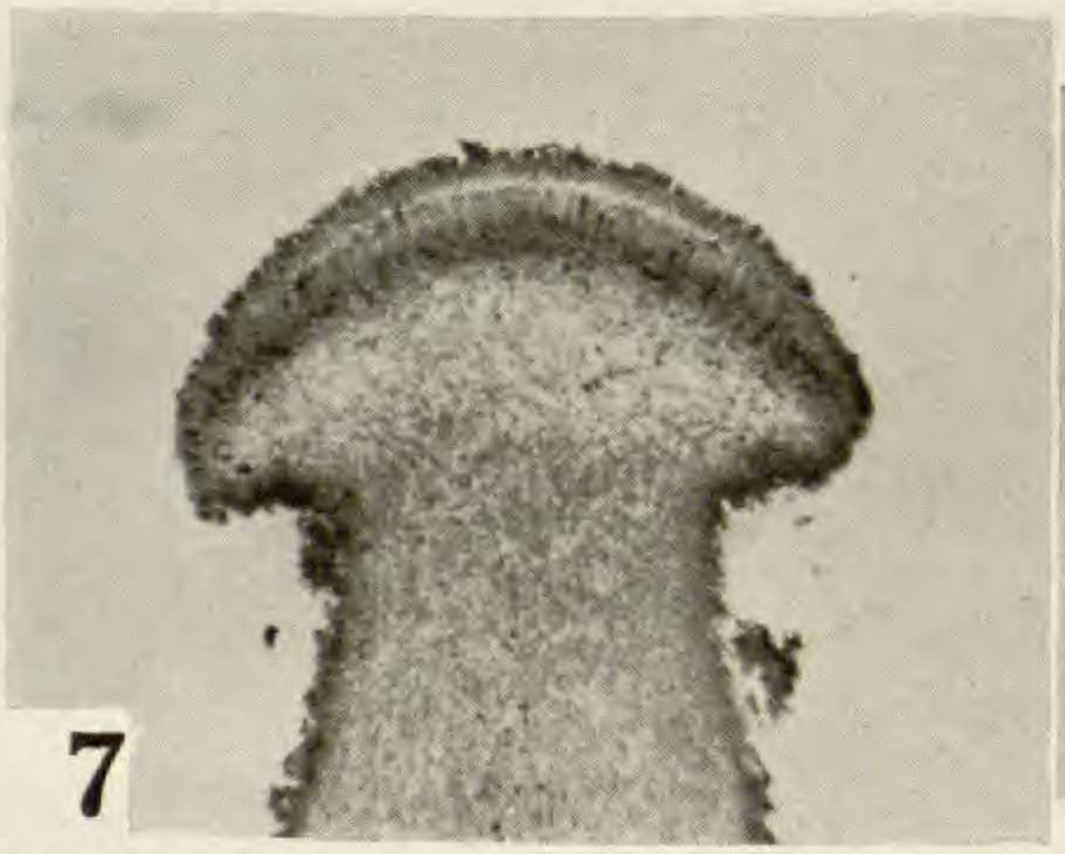

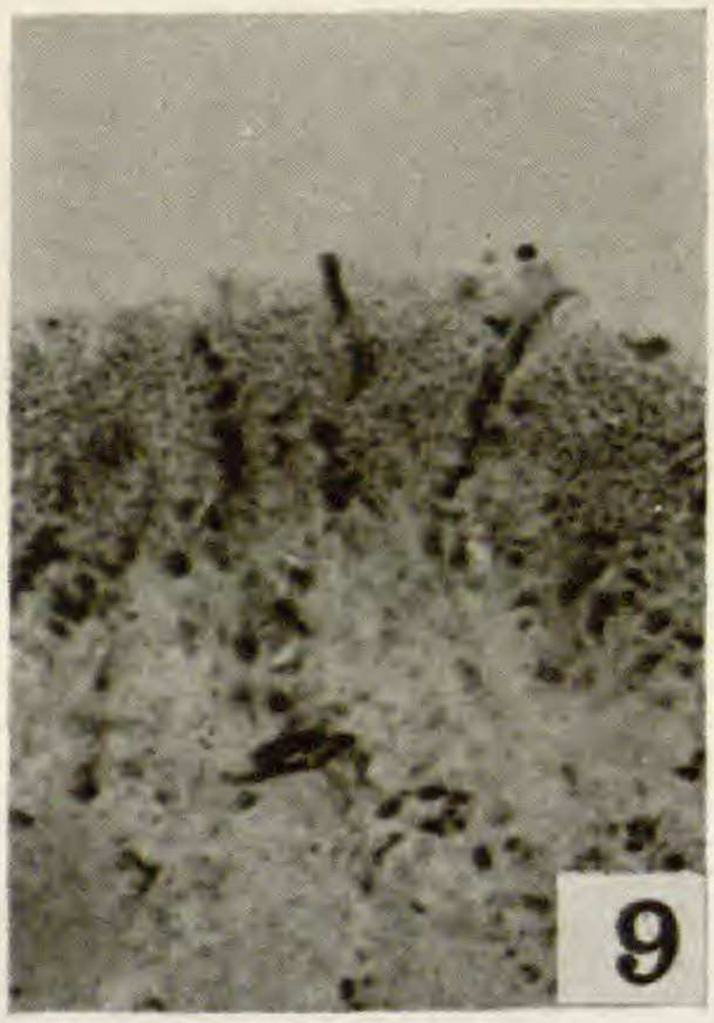

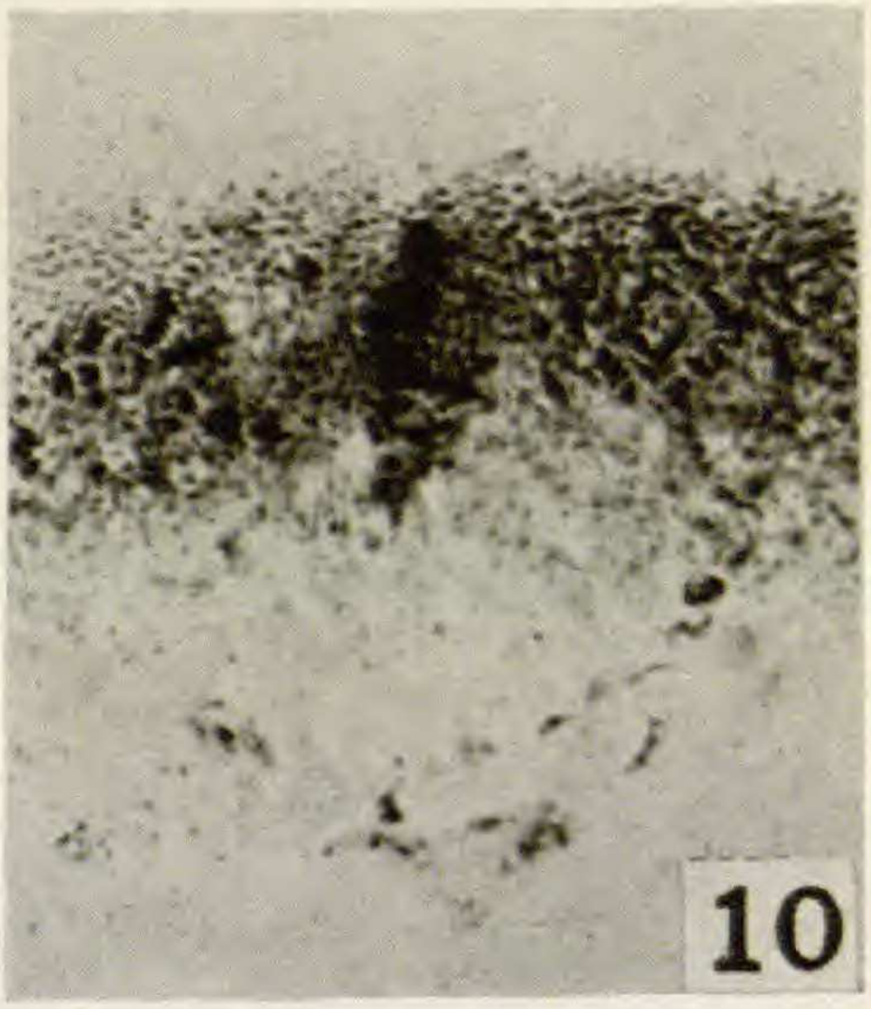

DUFF on GEOGLOSSACEAE